# Spectroscopic Evidence for Ligand-induced Conformational Change in $NADP^+$:Isocitrate Dehydrogenase*

**Conformational changes induced by binding of ligands to cytosolic $NADP^+$-specific isocitrate dehydrogenase from lactating bovine mammary gland were assessed using circular dichroism and fluorescence techniques. The secondary structure of isocitrate dehydrogenase, as monitored by CD spectra in the far-UV region, is unaltered by enzyme-ligand interactions; in contrast, dramatic changes occur in the near-UV region (270–290 nm) assigned to tyrosine and/or solvent-exposed tryptophan residues. Both the coenzyme analog, 2′-phosphoadenosine 5′-diphosphoribose, and NADPH have an effect on the CD spectrum which is opposite to that produced by metal complexes of either isocitrate or citrate. A CD band at 292 nm assigned to ~2 tryptophan residues in a hydrophobic environment is unchanged by binding of substrate or coenzyme. Approximately 30% of the intrinsic fluorescence of isocitrate dehydrogenase, corresponding to ~2 tryptophan residues, is not quenched by acrylamide in the absence of 6.3 M guanidine hydrochloride and remains unquenched in the enzyme-substrate complex. The constancy in the proportion of buried and exposed tryptophan residues implicates tyrosine in the observed near-UV CD spectral changes. Since binding of ligands does not influence quaternary structure (Seery, V. L., and Farrell, H. M., Jr. (1989) *Arch. Biochem. Biophys.* 274, 453–462), activation of isocitrate dehydrogenase may be related to a substrate-induced conformational transition.**

The cytosolic isozyme of NADP-specific isocitrate dehydrogenase (*threo*-$D_S$-isocitrate:$NADP^+$ oxidoreductase (decarboxylating) EC 1.1.1.42) is a major source of reducing equivalents in the form of NADPH for biosynthetic reactions in several animal tissues including mammary gland, adrenal gland, and liver. In lactating ruminant mammary tissue, isocitrate dehydrogenase (IDH)[1] provides NADPH for fatty acid and cholesterol synthesis (Moore and Christie, 1981; Farrell *et al.*, 1987). The mechanism(s) that control the activity of the enzyme *in vivo* is (are) unknown. The purified enzymes from bovine liver (Carlier and Pantaloni, 1973) and lactating mammary gland (Farrell *et al.*, 1990) exhibit a distinct lag time in stopped-flow kinetic experiments which is abolished by preincubation with the substrate, a complex (1:1) of a divalent metal ion and isocitrate. Possible mechanisms of activation of IDH by its substrate include a change in the conformation and/or aggregation state of the enzyme. Using analytical ultracentrifugation and kinetic methods we failed to observe changes in the oligomeric structure of the enzyme from mammary gland as a consequence of ligand binding (Seery and Farrell, 1989). In this report we use circular dichroism in the near- and far-UV as well as fluorescence spectroscopy to monitor the formation of enzyme-substrate and enzyme-coenzyme complexes. The binding of either the substrate or the coenzyme perturbs the environment of tyrosine and/or tryptophan residues of IDH. This observed modification of the tertiary structure of the enzyme is not accompanied by significant alteration of its secondary structure. The results suggest that activation of the soluble IDH is related to subtle changes in conformation induced by the substrate.

## EXPERIMENTAL PROCEDURES

*Materials*—The isolation of isocitrate dehydrogenase from bovine mammary gland has been described (Farrell, 1980). The preparations used in this work were homogeneous as judged by sodium dodecyl sulfate-gel electrophoresis and had specific activities ranging from 46 to 54 μmol/min/mg protein. An absorbance index (0.1%, 1 cm) of 1.37 at 278 nm (Seery and Farrell 1989) was used to measure concentration of protein for spectral analyses. The chemicals and biochemicals used in this study as well as the method for calculating the concentration of free metal have been previously described (Seery and Farrell, 1989). The term "isocitrate" used throughout this report refers to the *threo*-$D_s$(+) form of isocitrate. For one preparation of the enzyme the following treatments were added to the isolation procedure before chromatography on Sephacryl S-200 to insure complete removal of any bound nucleotide: (*a*) 0.2 M NaCl followed by desalting using Sephadex G-25, and (*b*) 0.5 mM manganous-isocitrate. The ratio of absorbances (340:280 nm) was 0.03 for this preparation and <0.1 for all others.

*Amino Acid Analysis*—Amino acid analyses were carried out as previously described (Thompson *et al.*, 1989). The protein was *S*-carboxymethylated by the method of Schechter *et al.* (1973) and similarly analyzed to obtain an estimate of the cystine content. The residues per subunit were calculated from molar ratios using phenylalanine equal to 18. The number of tryptophan residues per subunit was estimated by the method of Edelhoch (1967) from the absorbance of solutions of IDH dissolved in 6.3 M guanidine hydrochloride containing 0.01 M Tris·Cl (pH 7.4) and 0.03 M KCl.

*Circular Dichroism Studies*—Protein samples for CD measurements were dialyzed for 12–16 h *versus* buffer (0.02 M Tris-Cl, 0.04–0.08 M KCl, pH 7.4) and filtered through Nucleopore membrane filters (0.4 μm) before use. Concentrated ligand solutions were added directly to the cuvettes from a microsyringe; total dilution of the enzyme did not exceed 4%. Circular dichroism spectra were recorded

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[1] The abbreviations used are: IDH, isocitrate dehydrogenase; PADPR, 5′-phosphoadenosine 3′-diphosphoribose.

TABLE I

*Amino acid content of bovine mammary gland NADP⁺:isocitrate dehydrogenase*

Amino acid analyses were carried out as described under "Experimental Procedures." The timed hydrolyses were obtained by duplicate analysis at 48 h and triplicate analyses at 24 and 72 h. Serine content was estimated by extrapolation back to zero time, glycine was from the 72-h samples, and cystine was determined on the *S*-carboxymethylated protein. All other values represent pooled data and have an average standard error of 1.5% except for proline and methionine at 4%.

| Amino acid | Mol % | Residues per 49,500 | Amino acid | Mol % | Residues per 49,500 |
|---|---|---|---|---|---|
| Cys(Cm) | 1.5 | 7 | Met | 3.0 | 13 |
| Asp | 10.2 | 44 | Ile | 6.4 | 28 |
| Thr | 5.3 | 23 | Leu | 7.0 | 30 |
| Ser | 5.7 | 24 | Tyr | 4.4 | 19 |
| Glu | 11.7 | 50 | Phe | 4.2 | 18 |
| Pro | 3.6 | 15 | Lys | 8.7 | 38 |
| Gly | 8.3 | 36 | His | 2.6 | 11 |
| Ala | 7.4 | 32 | Trp | | 8 |
| Val | 6.3 | 27 | Arg | 3.7 | 16 |

on a Jasco model J-41C[2] spectropolarimeter at ambient temperature (21 ± 1 °C). The far-UV region of the spectrum (195–240 nm) was measured in a 0.05-cm cylindrical cell using samples containing ~0.4 mg/ml protein. In the near-UV region (245–320 nm) the data processor of the instrument was used to average a total of 8 or 16 scans; spectra were accumulated in 1-cm cuvettes containing 0.4–0.7 mg/ml protein. Solvent base lines were measured under the same conditions as that of the sample. Spectra obtained from the model J-41C for both sample and solvent were converted to digital form and entered into a Modular Computer Systems model III computer for smoothing and processing. Spectra presented in the figures are corrected for the solvent contributions (including all additives) and are expressed as molar ellipticity in the near-UV and mean residue ellipticity in the far-UV. Values of 112 for the mean residue molecular weight and 0.736 ml/g for the partial specific volume were calculated from the amino acid composition (see above). Molar ellipticity values were computed on the basis of a molecular weight of 49,500 for the subunit of IDH (Seery and Farrell, 1989). The content of each type of secondary structure was estimated from the far-UV CD spectrum (200–250 nm); reference spectra were either those of Chen *et al.* (1974) or Stone *et al.* (1985).

*Fluorescence Studies*—Fluorescence spectra were recorded at 25.0 ± 0.1 °C using a Perkin-Elmer MPF-44E spectrofluorometer in the ratio mode with slit widths of 5 nm. Samples of IDH had an absorbance ≤ 0.05 at the exciting wavelength (295 nm). Fluorescence intensities observed during the titrations of IDH with acrylamide (7.5 M) were corrected for optical screening and dilution of the enzyme (≤10%).

## RESULTS AND DISCUSSION

*Amino Acid Composition*—Results of amino acid analyses on samples of IDH are summarized in Table I. The addition of 6.3 M guanidine HCl to solutions of IDH brought about a shift in the wavelength of maximum absorbance from 278 to 276 nm but had no effect on the magnitude (corrected for dilution) of the absorbance at 280 nm. The value of $\epsilon$ (mM) calculated from the amino acid content and the molecular absorbances of tyrosine and tryptophan at 280 nm (Fasman, 1976) is 67 and agrees closely with the experimentally determined value of 66 (Seery and Farrell, 1989). With respect to its amino acid composition, mammary IDH is more closely related to other mammalian cytoplasmic enzymes than to the mitochondrial forms of IDH (Seelig and Colman, 1978).

*Far-UV CD of IDH*—The circular dichroism spectrum (200–250 nm) of IDH (——) is shown in Fig. 1. Theoretical

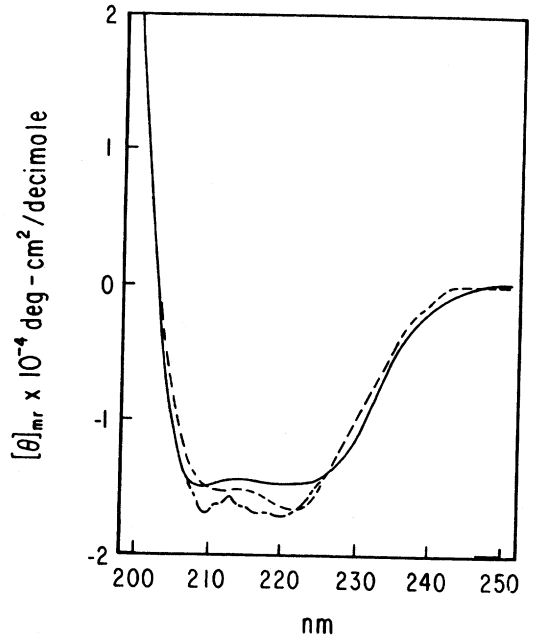


FIG. 1. **Far-UV circular dichroism spectrum of isocitrate dehydrogenase.** The concentration of IDH was 6.2 μM in a buffer containing 0.02 M Tris·Cl, 0.05 M KCl, and 0.1 mM dithiothreitol at pH 7.4. The optical path was 0.05 cm. The spectrum was not altered by the addition of 1.0 mM *threo*-$D_s$(+)-isocitrate and 620 μM $MnSO_4$. The levels of free $Mn^{2+}$ and the manganous-isocitrate complex are 280 and 240 μM, respectively. ——, experimental data; – –, theoretical fit using the basis sets of Stone *et al.* (1985); – – –, theoretical fit using the basis sets of Chen *et al.* (1974).

curves which closely fit the experimental data are generated by assuming the following content of secondary structure: 1) (– – –) 52% α-helix and 22% β-form (Chen *et al.*, 1974) or 2) (– –) 41% α-helix, 32% β-form, and 13% β-turn (Stone *et al.*, 1985). The theoretical curve generated using the method of Stone *et al.* (1985) fits the experimental data more closely from the 209–220-nm range. The far-UV spectrum of mammary gland IDH resembles those previously reported for the cytosolic (Carlier and Pantaloni, 1973) and mitochondrial (Mas and Colman, 1985) forms of the enzyme. The addition of a saturating concentration (see below) of the manganous-isocitrate complex failed to bring about any detectable change in the spectrum of mammary gland IDH. Apparently, binding of the substrate does not alter the secondary structure of the enzyme.

*Near-UV CD of IDH*—The near-UV CD spectrum of IDH (Fig. 2) exhibits three main features: a positive band at ~292 nm, a negative band at ~278 nm, and a broad positive band centered at ~260 nm. The composite spectrum shown in Fig. 2 was obtained by averaging the results of three experiments conducted with different preparations of the enzyme. *Error bars* represent the range of observed values at selected wavelengths. The extent of the deviations indicate that the positions and intensities of the three CD bands are essentially constant. The value of the molar ellipticity at 278 nm is (−2.3 ± 0.3) × $10^4$ degrees cm$^2$/dmol. Addition of sodium dodecyl sulfate, a denaturant that disrupts tertiary and quaternary structure of proteins, abolishes the bands at 278 and 292 nm. These bands arise from interactions of aromatic residues with the local environment in the native enzyme (Strickland, 1974).

The near-UV CD spectrum of IDH bears a strong resemblance to the spectrum of azurin (Finazzi-Agro *et al.*, 1973), which contains 2 tyrosine and 6 phenylalanine residues, as

[2] Mention of brand name does not constitute endorsement of this product over any of a similar nature by the United States Department of Agriculture.

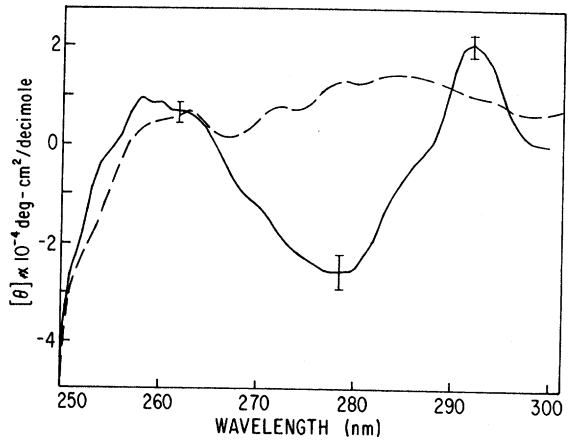


FIG. 2. **Near-UV circular dichoism spectrum of isocitrate dehydrogenase with and without sodium dodecyl sulfate.** The concentration of IDH ranged from 7.8 to 12.4 $\mu$M; the buffer contained 0.02 M Tris·Cl, 0.08 M KCl at pH 7.4. ——, no additions; – –, + 1 mM sodium dodecyl sulfate. *Error bars,* range of observed values at selected wavelengths.

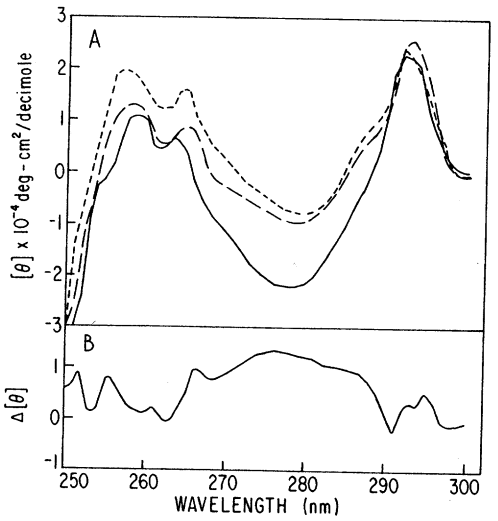


FIG. 3. *A,* near-UV circular dichoism spectrum of isocitrate dehydrogenase in the presence and absence of manganous-isocitrate The enzyme concentration was 12.4 $\mu$M in a buffer containing 0.02 M Tris·Cl and 0.08 M KCl at pH 7.4 with the following additions: ——, none; – –, 170 $\mu$M *threo*-$D_s$(+)-isocitrate and 510 $\mu$M $MnSO_4$; - - -, 500 $\mu$M *threo*-$D_s$(+)-isocitrate and 510 $\mu$M $MnSO_4$. The level of free $Mn^{2+}$ was constant at 280 $\mu$M while the concentration of manganous-isocitrate varied were from 40 (– –) to 120 $\mu$M (- - -). *B,* difference circular dichoism spectra. Difference curves were obtained by subtracting the spectrum of a mixture of IDH and 40 $\mu$M $Mn^{2+}$-isocitrate from the spectrum of the enzyme alone.

well as a single tryptophan buried within the interior of the molecule in a nonpolar region (Norris *et al.,* 1983). The strong positive band at 292 nm in the CD spectrum of azurin can be assigned to the 0 – 0 $^1L_b$ transition of the single tryptophan residue, while the band at 285 nm appears to be the 0 + 850 $cm^{-1}$ $^1L_b$ transition superimposed on a complex negative band arising mainly from tyrosine (Strickland, 1974). The absorption spectrum of azurin exhibits well resolved fine structure at 292 nm (Finazzi-Agro *et al.,* 1970) which is characteristic of tryptophan derivatives in nonpolar solvents (Strickland *et al.,* 1971). The ratio Tyr:Trp in IDH is also approximately 2:1. The positive band at 292 nm in the CD spectrum of IDH can be unequivocally assigned to the 0 – 0 $^1L_b$ transition of tryptophan; most likely, the 0 + 850 $cm^{-1}$ $^1L_b$ transition is represented by the shoulder at about 285 nm on the prominent negative CD band. The magnitude of the molar ellipticity at 292 nm ((1.9 ± 0.2) × $10^4$ degrees $cm^2$/dmol) indicates that the band represents at least 2 tryptophan residues (Strickland *et al.,* 1974). The presence of tryptophan residues in a nonpolar environment is confirmed by the shoulder in the absorption spectrum of IDH at about 292 nm (data not shown). The $^1L_a$ band from tryptophan, which often appears in the region from 295 to 305 nm, can not be distinguished. Although the strong negative CD band may be complex, both the position of the extremum and the similarity to the spectrum of azurin suggest that 0 + 850 $cm^{-1}$ transitions of several tyrosine residues are involved.

*Interaction of IDH with Substrate*—Binding of manganous-isocitrate by the enzyme dramatically alters the near-UV CD spectrum of IDH (Fig. 3*A*). The negative band at 278 nm is greatly reduced in the presence of the substrate, but there is relatively no change in the positive band at 292 nm. The spectral transition at 278 nm is complete at 41 $\mu$M manganous-isocitrate. No further decrease in molar ellipticity is observed when the concentration of manganous-isocitrate is increased to 123 $\mu$M and to 500 $\mu$M (not shown). The position and the intensity of the band at 292 nm are relatively unchanged during the titration with manganous-isocitrate indicating the absence of base-line drift as the 8–16 scans were recorded. The CD spectrum of the enzyme was not modified by the addition of 600 $\mu$M isocitrate alone (not shown). The difference CD spectrum which is generated by subtracting the spectrum of the enzyme from that of the saturated enzyme-substrate complex (Fig. 3*B*) exhibits a broad band extending from 270 to 290 nm. The maximum difference is observed at about 277 nm and is 1.2 × $10^4$ degrees $cm^2$/dmol. A difference CD spectrum (270–290 nm), similar in nature to that of Fig. 3*B,* was also generated by the addition of manganous-isocitrate (250 $\mu$M) to an enzyme preparation which was exposed to substrate (0.5 mM) during one step of the isolation procedure. Analysis of nine spectra from three different preparations of IDH at concentrations of manganous-isocitrate from 41 to 500 $\mu$M reveals that the maximum spectral change occurs between 276 and 283 nm; the magnitude of the change, (1.5 ± 0.4) × $10^4$ degrees $cm^2$/dmol, represents a 66 ± 20% decrease in molar ellipticity. Assuming that the enzyme is 90% saturated with the substrate at a concentration of 41 $\mu$M, the $K_d$ value for the interaction of IDH with metal-isocitrate is estimated to be 2.6 $\mu$M. The estimated $K_d$ value is approximately equal to the $K_m$ of 3.0 $\mu$M determined under assay conditions (Farrell, 1980).

The difference CD spectrum generated by the binding of manganous-isocitrate to IDH is similar to those reported for ligand binding to enzymes and attributed mainly to altered contributions from tyrosyl side chains (Fretto and Strickland, 1971; Griffin *et al.,* 1972). The simplest explanation for the reduced optical activity from 270 to 290 nm in the complex of IDH with its substrate is a change in the sign of a CD band from negative to positive for 1 or more tyrosine residues. Studies with model compounds have shown that both the sign and the magnitude of the $^1L_b$ band of tyrosine are sensitive to the orientation of the aromatic ring with respect to the remainder of the molecule (Snow and Hooker, 1975). Since the intensity and wavelength position of the band at 292 nm remains constant, the environment of the buried tryptophans of IDH is not influenced by the formation of the enzyme-substrate complex. In addition, since the two vibronic CD bands from the $^1L_b$ electronic transition have the same sign (Strickland *et al.,* 1969), the contribution of the 0 + 850 $cm^{-1}$ $^1L_b$ band from tryptophan to the dicroism below 290 nm remains unchanged. The involvement of other unresolved

TABLE II

*Effect of ligands on the molar ellipticity at 278 nm in the near-UV CD spectrum of IDH*

| Experiment | Ligand(s) | Conc. | ME[a] ($10^{-4}$) |
|---|---|---|---|
| | | *mM* | |
| 1 | None | | −2.6 |
| | $Mn^{2+}$-citrate | 1.0 | −1.9 |
| | Above + NADPH | 0.01 | −2.3 |
| | NADPH | 0.01 | −4.0 |
| 2 | None | | −2.1 |
| | $Mn^{2+}$-citrate | 5.0 | −1.6 |
| 3 | None | | −2.0 |
| | PADPR | 0.01 | −2.4 |
| | | 0.02 | −3.0 |
| | $Mn^{2+}$-isocitrate | 0.12 | −0.7 |
| | Above + PADPR | 0.01 | −1.1 |

[a] Molar ellipticity (ME) values at 278 nm are averages obtained from 8 to 16 scans per sample and are expressed in degrees $cm^2$/dmol. Other details are given under "Experimental Procedures."

tryptophan CD bands to spectral transitions below 290 nm in the enzyme-substrate complex can not be excluded.

*Interaction of IDH with Coenzymes and Metal-Citrate*—Like manganous-isocitrate, the binding of either a coenzyme or a substrate analog induces changes in the near-UV CD spectrum (270–290 nm) of IDH which exhibit a maximum close to 278 nm. Molar ellipticity values (278 nm) observed in three experiments with and without various ligands are summarized in Table II. Manganous-citrate, which can replace the substrate as an activator of IDH, mimics the influence of the substrate (Farrell *et al.*, 1990). At 1 mM analog (Table II, *exp. 1*), negative molar ellipticity is decreased by ~18%; while at 5 mM (Table II, *exp. 2*), the band is decreased by ~35%. The maximum difference CD which was generated by the addition of the analog was $7 \times 10^3$ degrees $cm^2$/dmol or about half of the value observed with manganous-isocitrate. NADPH, whose effect is opposite to that of the substrate, greatly increases the magnitude of the negative band when compared to the spectrum of the enzyme alone (Table II, *exp. 1*). The difference CD produced by NADPH (11 μM) has a value of $-1.4 \times 10^4$ degrees $cm^2$/dmol. The spectrum obtained with mixtures of NADPH (11 μM) and manganous-citrate (1 mM) is nearly identical to that obtained with the substrate analog alone (Table II, *exp. 1*) suggesting that NADPH may not bind to the enzyme-analog complex. The reduced coenzyme is excluded from its binding site on the enzyme from beef liver by magnesium-isocitrate (Carlier and Pantaloni, 1976). The cofactor analog, 5′-phosphoadenosine 3′-diphosphoribose (PADPR) which lacks a nicotinamide ring, acts like NADPH to increase the magnitude of the negative band at 278 nm by ~20% at 8 μM and ~50% at 16 μM (Table II, *exp. 3*). Binding of PADPR to the enzyme partially reverses the effect of the substrate on the negative band. The difference CD at 278 nm which is generated by the addition of manganous-isocitrate (120 μM), has a value of $1.3 \times 10^4$ degrees $cm^2$/dmol in the absence of PADPR, as compared to $9 \times 10^3$ degrees $cm^2$/dmol in its presence. In the region below 268 nm, the effect of mixtures of PADPR and manganous-isocitrate is the sum of their individual contributions. This observation suggests that the substrate and the coenzyme analog can be bound simultaneously to the enzyme.

*Fluorescence Studies*—The possible role of tryptophan residues in enzyme-substrate interactions was further investigated by fluorescence techniques. The exposure of tryptophan residues in a protein can be quantitated from the quenching of tryptophan fluorescence by acrylamide in the presence and absence of a denaturant such as guanidine hydrochloride. The

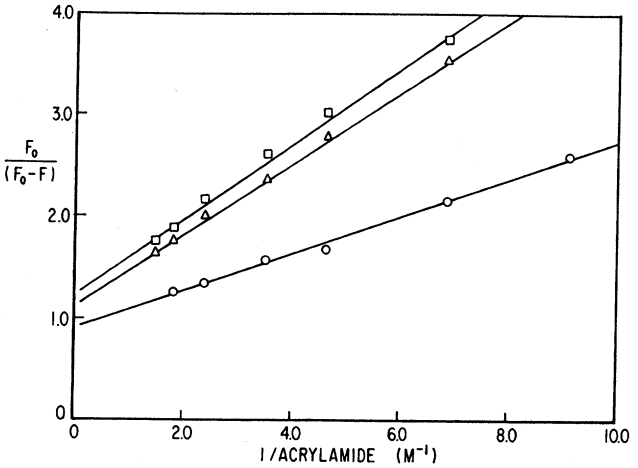


FIG. 4. **Fluorescence quenching of tryptophan residues in isocitrate dehydrogenase by acrylamide.** The buffer contained 10–20 mM Tris·Cl and 40–80 mM KCl at pH 7.4 with the following additions: none (△), 150 μM $MnSO_4$ and 500 μM *threo*-$D_s$(+)-isocitrate (□), and 6.3 M guanidine hydrochloride (○). The concentrations of free $Mn^{2+}$ and the manganous-isocitrate complex (□) were 80 and 40 μM, respectively.

emission maximum of the tryptophan fluorescence in IDH (336 nm) is blue-shifted relative to that of the denatured protein in 6.3 M guanidine·HCl (350 nm) indicating that a significant fraction of the estimated 8 tryptophan residues are shielded from the aqueous medium. Fig. 4 compares the quenching data for native IDH, the enzyme-substrate complex, and the unfolded protein according to the modified form of the Stern-Volmer equation (Lakowicz, 1983) as follows.

$$\frac{F_o}{F_o - F} = \frac{1}{f_a K_q [Q]} + \frac{1}{f_a}$$

$F_o$ and $F$ are the fluorescence intensities in the absence and presence of quencher ($Q$), $K_q$ is the quenching constant, and $f_a$ is the fractional quenching of tryptophan fluorescence. At infinite quencher concentration, 1/[$Q$], fluorescence is observed only from the inaccessible tryptophan residues. Values of $f_a$ which are obtained from the reciprocals of the intercepts on the abcissa are 0.79 ± 0.03 for the native IDH, 0.75 ± 0.02 for the enzyme-substrate complex, and 1.11 ± 0.05 for the denatured protein. In addition, the slopes of the plots obtained with (0.34 ± 0.03) and without (0.30 ± 0.04) substrate are similar. Thus, within the limits of experimental error, the fraction of exposed residues and the effective quenching constants are not altered by the presence of manganous-isocitrate. These findings are consistent with the failure of a saturating concentration of the substrate to appreciably alter either the magnitude or the $\lambda_{max}$ of emission. In guanidine HCl, $f_a$ is close to unity as expected for complete exposure of all tryptophan residues. The difference in the values of $f_a$ for native and denatured IDH (≥0.3) is an estimate of the fraction of unexposed residues. Assuming that the quantum yield of fluorescence for all residues is identical, the equivalent of 2 tryptophan residues appears to be shielded from the exterior by the tertiary structure of the enzyme. The results of both CD and fluorescence methods concur in predicting that approximately 2 of the 8 tryptophan residues in IDH are buried within the protein, are surrounded by nonpolar neighboring groups, and remain buried in the enzyme-substrate complex.

Changes observed in the near-UV CD spectrum of IDH arise from an altered environment of tyrosine and/or solvent-exposed tryptophan residues in the enzyme-substrate complex. The involvement of tryptophan residues in these inter-

actions can not be excluded. Nevertheless, the magnitude of the near-UV difference CD coupled with the failure to observe any changes in the acrylamide quenching of tryptophan fluorescence suggests that the optical activity of other chromophores, primarily tyrosine, are influenced by the binding of $Mn^{2+}$-isocitrate. Local interactions in the vicinity of a ligand-binding site may also be responsible for ellipticity changes in the absence of structural transitions (Strickland, 1974). The large changes accompanying the binding of ligands to IDH argue against a static mechanism. These observations lead us to propose that the tertiary structure of IDH is modified by the binding of substrate.

Based on the CD results in the near-UV, at least two conformational states may exist, in addition to that of the native enzyme: one induced by nucleotides and the other by substrates or substrate analogs. Transitions among these states occur without dissociation of the IDH dimer (Seery and Farrell, 1989). Different conformations have also been suggested for the binary complex of mitochondrial IDH with nucleotides as compared to the ternary complex of enzyme, nucleotide, and substrate (Reynolds *et al.*, 1978; Mas and Colman, 1985). We propose that the conformation stabilized by $Mn^{2+}$-isocitrate represents the activated (nonhysteretic) form of cytosolic IDH (Farrell *et al.*, 1990). Thus, similar conformational changes may be involved in the activation of both the mitochondrial and the cytosolic forms of the enzyme.

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